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CHRONIC DISABLING DERMATOSES

Annual Progress Report

by

Albert M. Kligman, M.D.

and

Richard R. Marples, B.M.

September 1969

(For the period 1 July 1968 to 31 May 1969)

U. S. Army Medical Research and Development Command

Washington, D. C. 20315

Annual Report to the Commission on Cutaneous Diseases
of the Armed Forces Epidemiological Board

Contract No. DA-49-193-MD-2137 ✓

University of Pennsylvania

Philadelphia, Pennsylvania 19104

SEP 16 1969

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Summary

A method of quantitating the desquamating layers of human skin (corneocyte count) has been applied to normal skin and lesions of psoriasis, tinea versicolor and dandruff. The corneocyte count is an objective measure of severity. Bacteria can also be studied.

The bacteriology of acne lesions and the importance of Corynebacterium acnes in the production of free fatty acids have been studied.

Methods for evaluating systemic and topical antibacterial agents have been refined.

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1. Studies of the desquamating portion of human stratum corneum:

By modification of the detergent scrub technique of Williamson and Kligman the keratinized cells of the desquamating layers can be quantitatively removed and stained with Huckers crystal violet and basic fuchsin. The mechanically dispersed suspension can be counted in a hemocytometer, examined as a wet mount or air dried for measurement of cell size.

This technique has been applied to normal areas and to psoriatic lesions, lesions of tinea versicolor and to the scalp in dandruff.

Normal skin: Glabrous skin gives a count of 10^5 cells per sq. cm., plantar and scalp counts are higher and forehead is distinctly lower. More nucleated cells are seen on the forehead. The distribution of counts is skewed. Logarithmic transformation normalizes the curve so that for statistical purposes the geometric mean is used.

TABLE 1

Corneocyte Counts Per Sq. Cm. of Different Body Areas

Body Area	Number of Samples	Arithmetic Mean	Geometric Mean	Average % Nucleated
Forehead	84	82,000	61,800	9.9
Axilla	63	118,000	104,000	2.3
Arm	27	187,000	152,500	0.7
Palm	10	103,000	96,400	2.6
Abdomen	11	218,000	134,000	0.4
Thigh	26	133,000	124,000	2.0
Heel	8	319,000	228,000	0.2

Regional differences in cell sizes can be determined. Desquamating cells from the axilla and abdomen are larger than those from peripheral sites. This may be important in epithelial cell carriage of pathogenic bacteria.

The reproducibility of the method is shown in fig. 1 where the right and left sides of the forehead in 38 individuals are compared.

Figure 1

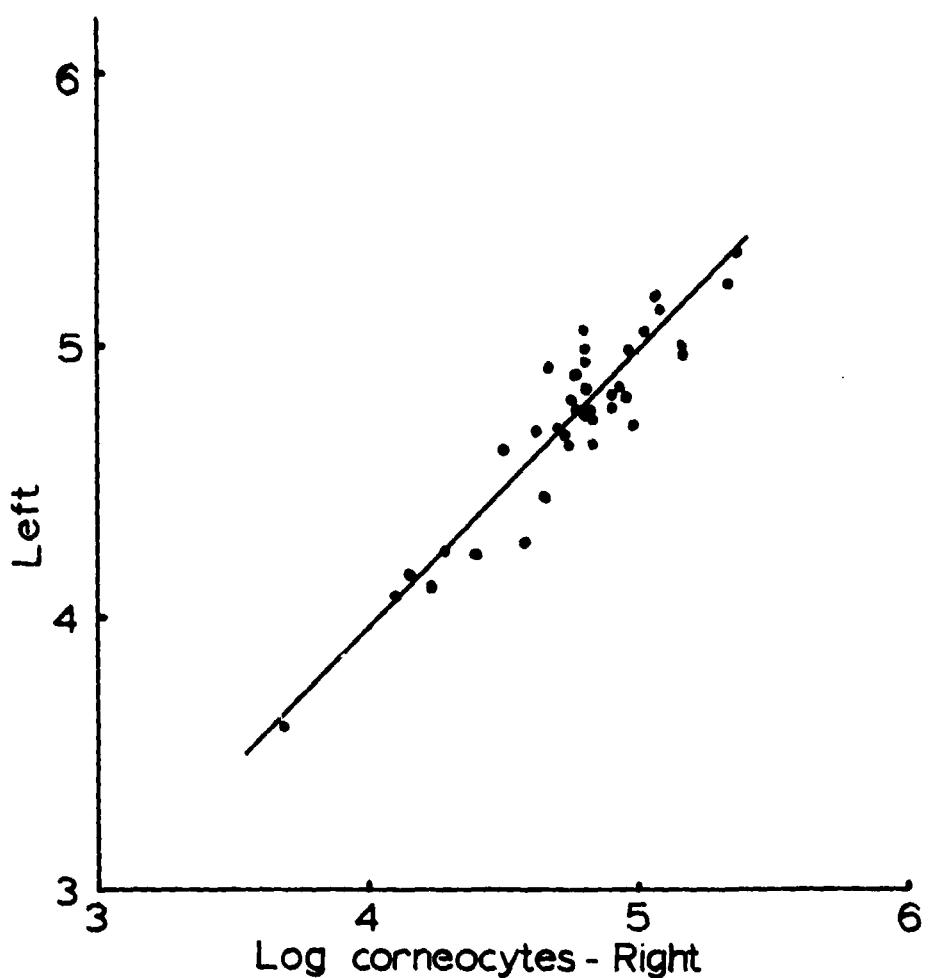
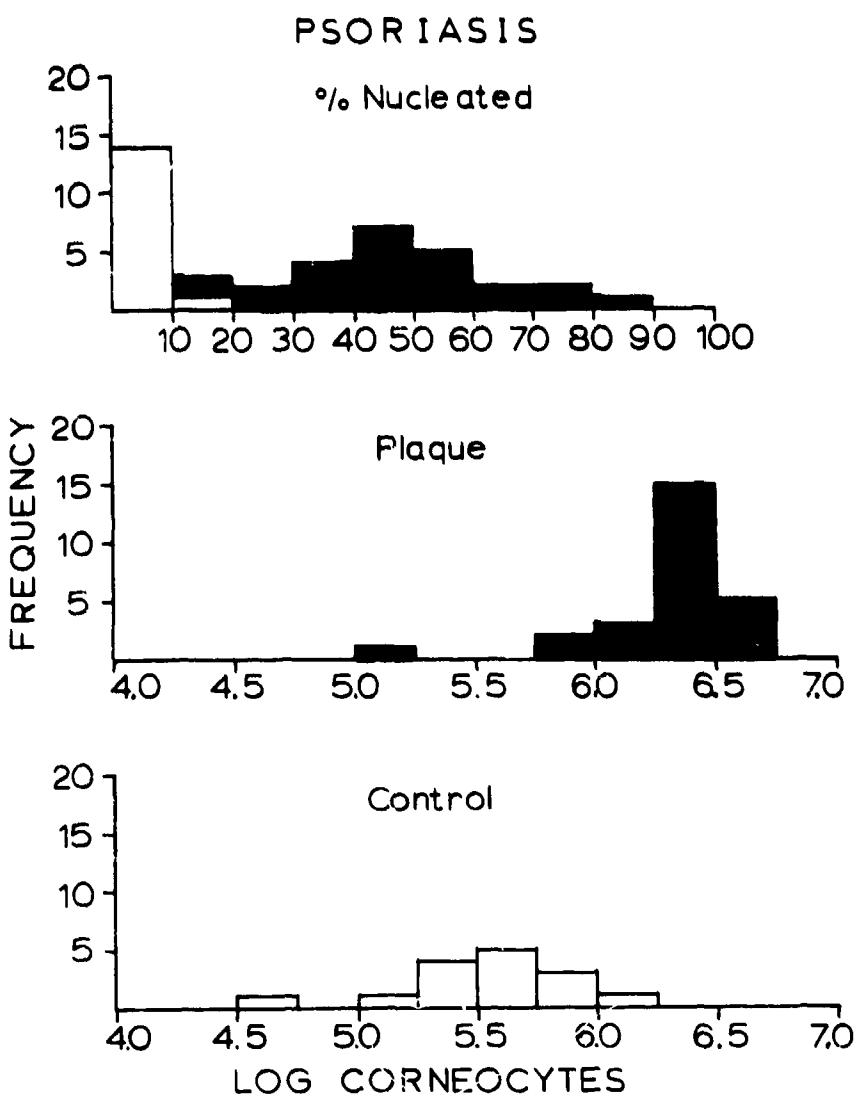


Fig. 1. Comparison of duplicate samples from the forehead

Psoriasis: Serial corneocyte counts were made on 26 lesions and 15 matching normal sites. Quantitative bacteriology was also performed on these samples. As expected, more cells were scrubbed off from the psoriatic scaling lesion than the clinically normal site.

Figure 2



The distribution of corneocytes from the normal site ranged from 30,000 to 2×10^6 with a peak at 300,000. The psoriatic sites ranged from 100,000 to 5.6×10^6 with a peak at 2.4×10^6 . The percentage of parakeratotic cells was also increased but varied from 10% to 80%. This index may be useful in following the recovery of the lesions. The poor quality of the psoriatic horny layer is shown by the proportion of the total cells removed which is present in the later scrubs. In the normal areas, of the cells removed in 5 minutes of scrubbing, 70% are removed by two minutes of scrubbing. In the psoriatic lesion, two minutes of scrubbing removes less than 60%.

The bacteriological results indicate that even in the psoriatic plaque, the organisms are superficial. In both the control and lesion series, 85-90% of all the bacteria removed are removed in the first two minutes of scrubbing. More bacteria were usually present on the lesion than the control site, but in those patients treated with hexachlorophene, the densities were lowered more in the psoriatic site. This question is of considerable importance in cross infection since topical antibacterial therapy seems to be more effective in the lesion than in the normal site.

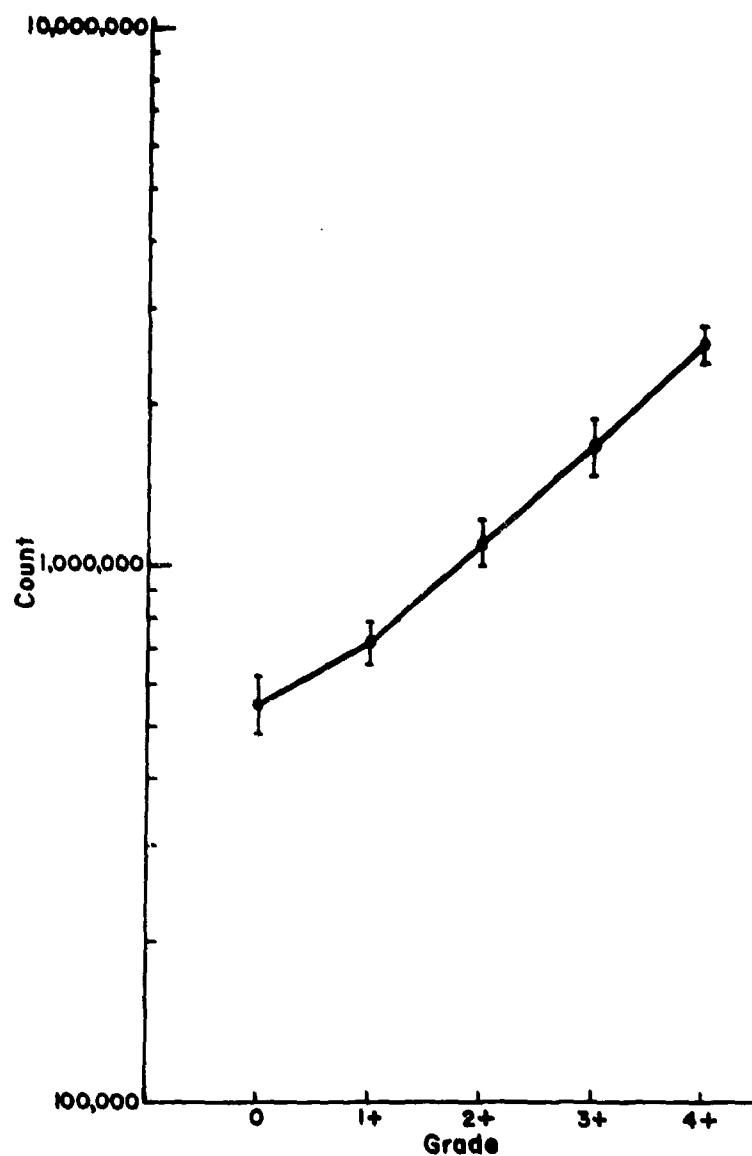
The kinds of bacteria present were usually the same as on normal skin, but in 8 of 20 patients, the lesion bore S. aureus.

Tinea versicolor: Nineteen subjects with tinea versicolor were studied using the corneocyte counting technique and quantitative bacteriology. Yeast cells and mycelial cells were also counted. Paired affected and clinically normal sites were sampled.

The geometric mean corneocyte count from the clinically involved sites was 390,000 compared to 114,000 for the control sites. Bacteriologically no difference was found between the two sites. In the lesion, the mean density of yeast forms was 193,000/ sq. cm. but the unaffected sites yielded 17,000/ sq. cm. The relative number of yeast cells to mycelial cells was 1:2.1 in the affected areas and 1:0.2 in the clinically normal areas. However, mycelial cells were present in all but 5 of the control samples.

Dandruff: The technique is a method very suitable for quantitation of dandruff in which clinical grading is difficult. A corneocyte count of less than 700,000 cells/ sq. cm. indicates no dandruff. Moderate dandruff has values between 900,000 and 1,400,000 while in severe dandruff the count lies above 1,500,000.

Figure 3



Conclusions:

1. The corneocyte count is a useful technique for quantitation of the desquamating layer of the human stratum corneum in health and disease.
2. The horny layer in psoriatic lesions is less coherent than in clinically normal sites and may carry *S. aureus* which is disseminated to the environment on the increased number of scales.
3. The utility of diminishing the carriage of *S. aureus* by hexachlorophene should be further studied.
4. The organism in tinea versicolor is not restricted to the clinically affected area.

2. Studies related to acne:

(a) Bacteriology of pustular acne:

Previous workers have shown that acne lesions of all types contain both Corynebacterium acnes and coagulase negative cocci. In recent years it has been felt that C. acnes is numerically more significant than the cocci. We studied 109 pustules in 27 patients and classified the cocci using the Baird Parker scheme.

Of the 109 pustules 80 contained C. acnes, 65 contained cocci, 11 contained gram negative rods, 9 contained lipophilic diphtheroids and 13 were sterile. If C. acnes and cocci acted synergistically, the frequency of isolation of the two should exceed that predicted from overall incidence.

TABLE 2

Incidence of Bacteria in Acne Pustules

	Observed		Expected	
	Pustules	%	Pustules	%
<u>C. acnes</u> alone	25	22.9	26.6	24.4
<u>C. acnes</u> + cocci	38	34.9	39.4	36.1
<u>C. acnes</u> + GNR	3	2.8	3	2.8
<u>C. acnes</u> , cocci GNR	5	4.6	4.4	4.0
<u>C. acnes</u> , cocci, lip. dip.	9	8.3	3.5	3.2
Cocci alone	13	11.9	14.3	13.1
Cocci + GNR	0	0	1.6	1.5
GNR alone	3	2.8	1.1	1.0
Other combinations	0	0	5.4	5.0
No organisms	13	11.9	9.7	8.9

This was not found, indicating a lack of synergism in the production of pustules by these organisms. The density of both organisms in pustules was very similar.

Table 3 shows the Baird Parker types of the 76 strains of gram positive cocci isolated from 65 pustules. Micrococcus isolates were uncommon. In the genus Staphylococcus, S. epidermidis (Types SII and SV) was by far the most common. S. aureus (Type S I) was identified on only two occasions.

TABLE 3
Baird Parker types of cocci from acne pustules

<u>Staphylococcus</u>		<u>Micrococcus</u>	
S I	2	M 1	1
S II	64	M 2	1
S IV	1	M 3	3
S V	3		
S VI	1		
	71		5

The frequency of S II is higher than would be expected from the flora of normal skin; also M 7 (Sarcina lutea) which is commonly found on normal skin was not recovered from these lesions. These findings confirm the assumption that S II strains are more virulent than other coagulase negative cocci.

In two patients large numbers of Enterobacter aerogenes were isolated from 7 pustules. These individuals appeared to have gram negative folliculitis (Publication 5).

(b) Bacteriology of acne comedones:

The bacteriology of comedones from 33 acne patients and 20 senile comedones has been studied. The results are incomplete but it is clear that in comedones of both types, C. acnes outnumbers coagulase negative cocci by at least three times and often 10 times. In horizontal serial frozen sections, we were able to demonstrate by culture and by histology that the cocci were mainly located in the orifice while the deeper portions contained C. acnes predominantly. This stratification may reflect availability of oxygen.

(c) Role of *C. acnes* in the production of free fatty acids

By means of in vitro systems both C. acnes and coagulase negative skin cocci have been shown to produce Tiases able to hydrolyse triglycerides with the liberation of free fatty acids. The relative importance of these two groups in liberating free fatty acids from sebaceous secretions in the living subject has not been studied.

By application of neomycin to the scalp for 4 weeks, we were able to reduce the aerobic density from 570,000 bacteria per sq. cm. to 3,000 while the density of C. acnes did not fall. A significant correlation between the percentage of free fatty acids in scalp sebum and the density of C. acnes was shown. This was not affected by treatment.

Figure 4

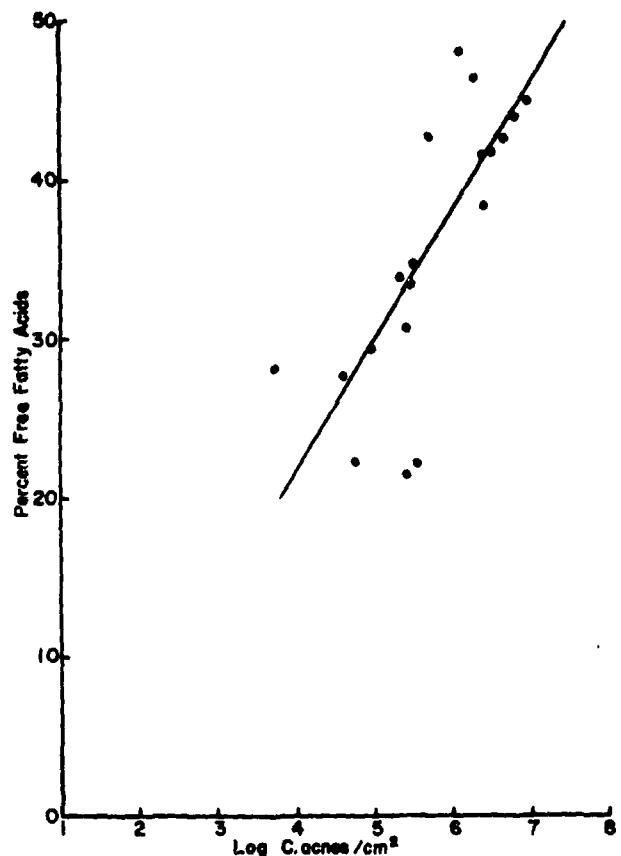
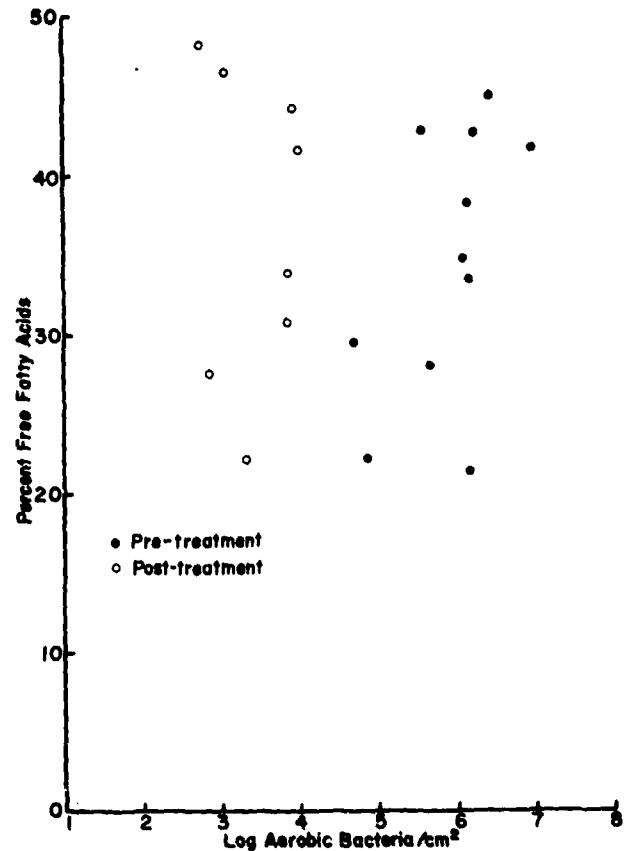


Figure 5



The original correlation of aerobes with percentage free fatty acids is abolished by treatment (fig. 5).

Conclusion:

The aerobic flora is not a significant source of lipolytic activity in vivo.

3. Studies of the effect of systemic antibiotics on the normal flora

Bacterial samples were taken before treatment, after one week and after three weeks treatment from both axillae and both sides of the forehead of five groups of six subjects. The treatments were tetracycline, 1 gm. daily, demethylchlortetracycline, 600 mgm. daily, doxycycline 200 mgm. daily, minocycline 200 mgm. daily and ampicillin, 1 gm. daily.

The results indicate that at this dosage,

1. demethylchlortetracycline and tetracycline are equivalent
2. minocycline may be slightly more active
3. doxycycline is least active of the tetracyclines but is still more active than ampicillin.

The data from these studies is still being processed and further longer term studies to include the scalp flora are envisaged.

4. Studies of experimental evaluation of topical antibacterials

Methods for evaluating antibacterial agents have been designed (Publication 7). Such tests include:

- a. The occlusion test. When forearm skin is occlusively sealed under Saran Wrap, the population of normal skin organisms increases enormously within 48 hours. Application of an effective bacteriostat just prior to occlusion will prevent the rise.
- b. The axilla test. By treating the axilla under quantitative bacteriological monitoring effectiveness and substantivity can be determined.
- c. The S. aureus test. Tape stripped skin or alcohol pretreated skin can be infected with *S. aureus* under occlusion. Test substances can be evaluated for both prophylactic and therapeutic actions.

Appendix I

Publications

1. Kligman, A. M. and Breit, R.: Identification of phototoxic drugs by human assay. *J. Invest. Derm.* 51: 90-99, 1968.
2. Willis, I. and Kligman, A. M.: Diagnosis of photosensitization reactions by the Scotch Tape provocative patch test. *J. Invest. Derm.* 51: 116-119, 1968.
3. Willis, I. and Kligman, A. M.: The mechanism of photoallergic contact dermatitis. *J. Invest. Derm.* 51: 378-384, 1968.
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5. Fulton, J. E., McGinley, K., Leyden, J. and Marples, R. R.: Gram-Negative Folliculitis in acne vulgaris. *Arch. Derm.* 98: 349-353, 1968.
6. Marples, R. R. and Kligman, A. M.: Growth of bacteria under adhesive tape. *Arch. Derm.* 99: 107-110, 1969.
7. Marples, R. R. and Kligman, A. M.: In vivo methods for appraising antibacterial agents. *T.G.A. Cosmetic Journal* 1: 26-33, 1969.
8. Marples, R. R.: Diphtheroids of Normal Human Skin. *B. J. Derm.* 81 (Supp. 1) 47-54, 1969.

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